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Human skin penetration of selected model mycotoxins

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ABSTRACT

Dermal exposure data for mycotoxins are very scarce and fragmentary, despite their widespread skin contact and hazard toxicity. In this study, the transdermal kinetics of aflatoxin B1 (AFB1), ochratoxin A (OTA), fumonisin B1 (FB1), citrinin (CIT), zearalenone (ZEA) and T-2 toxin (T-2) were quantitatively evaluated, using human skin in an *in vitro* Franz diffusion cell set-up. All mycotoxins penetrated through the skin, except for FB1, which showed concentrations in the receptor fluid below the LoD, resulting in a $K_p < 3.24 \times 10^{-6}$ cm/h. OTA showed the highest permeation ($K_p = 8.20 \times 10^{-4}$ cm/h), followed by CIT ($K_p = 4.67 \times 10^{-4}$ cm/h). AFB1 and ZEA showed lower permeability rates ($K_p = 2.11$ and 2.33×10^{-4} cm/h, respectively). T-2 was found to have the lowest permeability ($K_p = 6.07 \times 10^{-5}$ cm/h). From literature-based mycotoxin-concentrations, dermal contact surface, exposure time and apparent K_p's obtained in this study, the daily dermal exposure (DDE) in two industrial and one residential scenario was estimated. Dermal exposure to the DNA-reactive genotoxic carcinogenic AFB1 can lead to a health risk for agricultural workers which are exposed to a mycotoxin contaminated solution in a worst case situation. For all the other investigated mycotoxins, no significant health risk is calculated after dermal contact in neither agricultural nor residential environments.

1. Introduction

Mycotoxins are secondary metabolites produced by fungi (Braese et al., 2009). The worldwide contamination of these ubiquitous natural products in food, feed and environment, including indoor surfaces and particles, form a health risk for animals as well as humans (Zain, 2011). Over 400 mycotoxins are identified, but there are indications that thousands of mycotoxins exist (Nielsen and Smedsgaard, 2003). Very often, more than one mycotoxin is found on the contaminated substrate. The question arises to what extent these mycotoxins form a health risk (Creppy, 2002; Hussein and Brasel, 2001; Muro-Cacho et al., 2004; Peraica et al., 1999). Absorption of mycotoxins often occurs by ingestion of contaminated food, but can also be via inhalation or dermal exposure to air and dust containing mycotoxins. However, there are many uncertainties about the toxin fraction absorbed and hence, about the true impact of mycotoxin exposure via the different routes. Mayer et al. (2007) identified the research need of exposure assessment to mycotoxins. The health risk of mycotoxins was generally assessed via the oral route. Recently, the inhalation route is also gaining scientific interest (Halstensen, 2008; Hardin et al., 2009; Soroka et al., 2008; Tangni and Pussemier, 2007; Terr, 2009). The skin however, is almost unexplored as exposure route. Although the skin forms a natural barrier for exogenous compounds, the low molecular weight mycotoxins are lipid soluble, possessing appropriate properties for occupational as well as accidental skin penetration. Compared to the amount of identified mycotoxins and their health risk, to date, skin permeability data of mycotoxins is nevertheless quite limited but highly wanted (Degen, 2011). Supplementary Table S1 gives a literature overview of the skin related mycotoxin research. Anno end 2011, about 50, mostly fragmentary studies, have evaluated the (trans)dermal behaviour and resulted effects of less than 20 mycotoxins, mainly AFB1 and T-2 toxin. The majority of these studies, about 70%, describe *in vivo* animal methods, frequently using rats, mice, pigs and rabbits. Half of the remaining studies have also applied *in vitro* animal skin models and very often, quantitative kinetics are lacking. Therefore, in this study, the transdermal kinetics of mycotoxins were quantitatively evaluated using excised human skin in an *in vitro* Franz diffusion cell (FDC) set-up.

Abbreviations: AFB1, aflatoxin B1; AFB2, aflatoxin B2; CIT, citrinin; FB1, fumonisin B1; FB2, fumonisin B2; FDC, Franz diffusion cell; OTA, ochratoxin A; OTB, ochratoxin B; T-2, T-2 toxin; ZEA, zearalenone; SA, skin area; ED, exposure duration; EF, exposure frequency; EV, event frequency; t_{event}, event duration; BW, body weight; AT, averaging time; TDI, tolerable daily intake; NCRI, negligible cancer risk intake; NOAEL, no observed adverse effect level; LOAEL, lowest observed adverse effect level; BMD, benchmark dose.

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Table 1
Selected model mycotoxins for transdermal investigation.

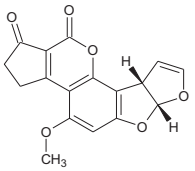
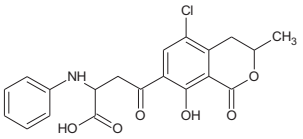
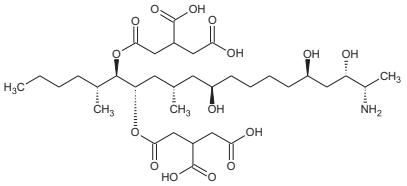
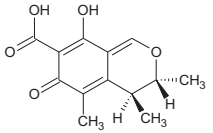
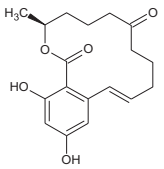
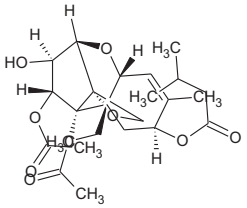
Class	Toxin	Structure	Derivative	MW	log *P*[a]	*In silico* K_p (cm/h)[b]	*In silico* toxicity data[c]	IARC classification[d]	Genotoxic literature data	EU maximal food levels (μg/kg)[e]
Aflatoxin	AFB1		Octaketide/furan derivative	312.27	1.16	1.57×10^{-4}	*Plausible* Chromosome damage, genotoxicity, mutagenicity *in vitro* in mammal Photoallergenicity, skin sensitization in mammal *Probable* Mutagenicity *in vitro* in bacterium Hepatotoxicity *in vitro* in mammal Carcinogenicity in mammal	Group 1	Genotoxic (Benford et al., 2010)	0.1–8.0
Ochratoxin	OTA		Pentaketide/ Isocoumarin derivative	403.81	4.27	7.10×10^{-3}	*Equivocal* Rapid prototypes: chromosome damage *in vitro* in mammal Rapid prototypes: nephrotoxicity, hepatotoxicity in mammal	Group 2B	Controversy for genotoxicity (Haighton et al., 2012; Kuiper-Goodman et al., 2010)	0.5–10.0
Fumonisin	FB1		Nonaketide	721.83	−0.78	2.10×10^{-8}	*Equivocal* Rapid prototypes: nephrotoxicity, hepatotoxicity in mammal	Group 2B	Controversy for genotoxicity (Ehrlich et al., 2002; SCF, 2000a)	200–2000[f]

Table 1 (*Continued*)

Class	Toxin	Structure	Derivative	MW	log *P*[a]	*In silico* K_p (cm/h)[b]	*In silico* toxicity data[c]	IARC classification[d]	Genotoxic literature data	EU maximal food levels (μg/kg)[e]
Citrinin type	CIT		Pentaketide	250.25	0.45	1.13×10^{-4}	*Equivocal* Chromosome damage *in vitro* in mammal *Plausible* Skin sensitization in mammal	Group 3	Controversy for genotoxicity (Flajs and Peraica, 2009)	–[na]
Resorcyl lactone	ZEA		Nonaketide/ Brefeldin type	318.36	3.66	8.68×10^{-3}	*Plausible* Skin sensitization in mammal	Group 3	Not genotoxic (Kuiper-Goodman, 1990; SCF, 2000b)	20–200
Trichothecene	T-2		Trichodermin type	466.52	1.45	2.93×10^{-5}	*Equivocal* Rapid prototypes: hepatotoxicity in mammal *Plausible* Mutagenicity *in vitro* in bacterium Chromosome damage *in vitro* in mammal Carcinogenicity, developmental toxicity, irritation of the eye, irritation of the skin, skin sensitisation in mammal	Group 3	Not genotoxic (SCF, 2001)	–[na]

[na] Not applicable.

[a] BioByte Corp., version 5.3.

[b] Potts and Guy, version 1.0.

[c] Derek Nexus 2.0 (Lhasa Limited).

[d] Group 1: proven to be human carcinogen, Group 2B: probable human carcinogen, Group 3: no carcinogenicity to humans.

[e] Commission Regulation (EC) No. 1881/2006.

[f] Sum of FB1 and FB2.

Model mycotoxins were selected based upon their toxicity, co-occurrence and toxicological interactions (Table 1). In most countries, aflatoxins (AF), ochratoxins (OT), fumonisins (F), deoxynivalenol (DON), zearalenone (ZEA), and patulin (PAT) are subjected to governmental regulation (Reddy et al., 2010). Aflatoxin B1 (AFB1), ochratoxin A (OTA) and fumonisin B1 (FB1) are proven to be the most toxic aflatoxin, ochratoxin and fumonisin, respectively. The International Agency for Research on Cancer (IARC) indicates that these mycotoxins are proven to be human carcinogen (AFB1) or probable carcinogens (OTA and FB1). Moreover, synergetic, additive and antagonistic interactions between pairs of these mycotoxins are observed at several target levels (Grenier and Oswald, 2011). Another regulated mycotoxin, the resorcyl lactone ZEA, shows no direct skin effect after topical application, yet ingestion results in exudative-necrotic inflammation of the skin (Juhasz et al., 2001; Vanyi et al., 1995). In addition, its interaction with OTA's nephrotoxic effects has been documented (Grenier and Oswald, 2011). The trichothecene T-2 toxin exhibits local as well as systemic effects after dermal absorption up to lethality (see Supplementary Table S1). Moreover, the co-occurrence with AFB1 (Coulombe, 1993) and interaction with other selected model mycotoxins (AFB1, OTA, and FB1) was reported (Grenier and Oswald, 2011). Therefore, T-2 toxin was included in our model set. Even though CIT is currently not regulated and is considered to be not carcinogenic to humans, its co-occurrence with OTA (Flajs et al., 2011; Flajs and Peraica, 2009; Pfohl-Leszkowicz and Manderville, 2007) and toxicological interactions with AFB1 and OTA (Grenier and Oswald, 2011) underpin our motivation to include this mycotoxin in the transdermal study as representative of the citrinin type. Moreover, very recently, one study reports the *in vivo* apoptosis in mouse skin via the intrinsic mitochondrial pathway (Kumar et al., 2011), indicating the relevance of CIT in dermal exposure framework.

2. Materials and methods

2.1. Chemicals and reagents

Six investigated mycotoxins (aflatoxin B1 (AFB1), ochratoxin A (OTA), fumonisin B1 (FB1), citrinin (CIT), zearalenone (ZEA) and T-2 toxin (T2)) (≥98.95% by HPLC) were supplied by Fermentek Ltd. (Jerusalem, Israel). LC–MS gradient grade methanol (MeOH) and acetonitrile (ACN) were purchased from Biosolve (Valkenswaard, The Netherlands). Ultrapure water (H_2O) was produced by a Milli-Q Gradient System (Millipore, Brussels, Belgium) or an Arium 611 purification system (Sartorius, Göttingen, Germany), resulting in ultrapure water of 18.2 MΩ cm quality. Ammonium bicarbonate (NH_4HCO_3) was obtained from Sigma–Aldrich (Bornem, Belgium). This latter was also the supplier of reference standards of ochratoxins A and B, aflatoxins B1 and B2, citrinin, T-2 and HT-2 toxins, zearalenone, zearalanone and fumonisins B1 and B2 (≥98.0% by TLC) used during the development of the analytical method. Ethanol (EtOH), denatured with up to 5% ether, was bought from Chem Lab (Zedelgem, Belgium). Acros (Geel, Belgium) provided *N,N*-dimethylformamide (DMF) and dichloromethane. 0.01 M phosphate buffered saline (PBS) was purchased from Sigma (St. Louis, MO, USA), while bovine serum albumin (BSA) was supplied by Merck (Darmstadt, Germany). Merck was also the supplier of formic acid and hydrochloric acid.

2.2. Analytical method

2.2.1. LC–MS/MS

For the quantitative mycotoxin analysis, a multi-LC–MS/MS method, based on Njumbe Ediage et al. (2012) was used. Briefly, the LC–UV/MS platform consisted of an Alliance HPLC 2695 apparatus (Waters, Milford, MA, USA) coupled to a Micromass Quattro LC triple quadrupole mass spectrometer, equipped with a Z-spray electrospray ionization (ESI) interface (Waters). An XBridge MS C_{18} column (3.5 μm, 150 mm × 2.1 mm) attached to an XBridge Sentry guard column (3.5 μm, 10 mm × 2.1 mm) (both Waters) was used for the chromatographic separation. The injection volume was 20 μL. The flow rate was set to 0.15 mL/min and the linear gradient used was as follows (where *A* = water/0.2 M ammonium bicarbonate (pH 10)/methanol (85/5/10, V/V/V) and *B* = water/0.2 M ammonium bicarbonate (pH 10)/methanol (5/5/90, V/V/V); *t* = 0–3 min, *A*/*B* (30/70, V/V); *t* = 3–6 min, *A*/*B* (10/90, V/V); *t* = 6–8 min, *A*/*B* (0/100, V/V); *t* = 8–16 min, *A*/*B* (30/70, V/V); *t* = 16–18 min, *A*/*B* (30/70, V/V). The mass spectrometer was operated in the positive electrospray ionization mode (ESI^+). Capillary voltage of 3.5 kV and argon collision gas of 1.2×10^{-3} mbar were applied. ESI source block and desolvation temperatures were set at 150 °C and 300 °C, respectively, while cone nitrogen and desolvation gas flows were 100 and 830 L/h, respectively. For increased sensitivity and selectivity, data acquisition was performed in selected reaction monitoring (SRM) mode. Data were acquired using Masslynx and Quanlynx software (Micromass, Manchester, UK). AFB2 was used as internal standard (IS) for AFB1, while OTB for OTA and CIT, FB2 for FB1, zearalanone for ZEA and HT-2 toxin for T-2 toxin.

2.2.2. Sample preparation

A clean-up procedure, using Oasis HLB™ SPE cartridges (Waters) was performed before LC–MS/MS analysis. The SPE cartridges were initially conditioned with dichloromethane/methanol (80/20, V/V), containing 50 mM formic acid, methanol, acidified water and finally non-acidified water. Subsequently, 200 μL of the PBS-based samples or 200 μL ethanolic samples (firstly evaporated to dryness and reconstructed in 200 μL 1% BSA in PBS) was quantitatively brought onto the SPE cartridge and washed with water. Elution of mycotoxins was performed by passing successively acetonitrile and methanol. The elute was evaporated under a gentle stream of nitrogen at 40 °C and reconstituted with 100 μL of injection solvent, which consisted of 80/20, V/V eluent A/eluent B. All samples underwent this sample preparation, except for the skin samples exposed to the mycotoxin mixture for 24 h, which first underwent an extraction with ethanol prior to this clean-up procedure.

2.3. Analytical skin-FDC developmental validation

The method has been validated. As the LoD values are important for the interpretation of current results, only these are given here: 1 ng/mL for AFB1 and CIT, 2.5 ng/mL for T-2, 5 ng/mL for OTA and 10 ng/mL for FB1 and ZEA. Two specific skin-FDC related method attributes are summarized hereafter.

2.3.1. Effect of skin components on the mycotoxin MS signal

A 1% BSA in PBS and a pure ethanol solution, both used as such or exposed to skin for 24 h to represent the over-contacting influence, were spiked with a mixture of the six targeted compounds and their internal standards (IS) at individual concentrations of 50 ng/mL. No matrix effect of the skin was found under these very harsh operational analysis (see Supplementary Table S2). Only for ZEA (in 1% BSA in PBS) and FB1 (in EtOH), an influence was observed, with an increased response of 33% and decreased response of 19%, respectively. However, as in an experimental FDC set-up, the skin was not fully soaked in the receptor fluid, but only exposed at a 0.64 cm^2 dermal surface area, no major influences were observed in the FDC experiments.

2.3.2. Stability of skin and transdermal samples

The FDC media are 1% BSA in PBS (receptor fluid samples), 70/30 (V/V) EtOH/H_2O (dose solutions) and EtOH exposed to blank skin for 12 h (skin extraction samples). Mixtures of the selected mycotoxins (50 ng of each mycotoxin/mL) in these three FDC media were stored for seven days at −35 °C and compared with corresponding freshly prepared solutions. PBS-based samples underwent the SPE cartridges clean-up, while the ethanol-based samples were evaporated to dryness at 40 °C and redissolved in the injection solvent. Table 2 summarizes the stability results in the three investigated media for each mycotoxin. Except for OTA in the skin exposed ethanolic solution, the mycotoxin concentrations were within the expected variability of the instrument performance (85–123%), which is acceptable for our FDC experiments: samples can thus be stored successfully for one week at −35 °C without stability issues ($p > 0.05$). However, as more than half of OTA in the skin exposed ethanolic solution has been degraded, skin extraction samples were analyzed immediately after sample preparation.

2.4. Franz diffusion cell experiments using human skin

Seen the extreme toxicity of some mycotoxins, it is ethically unacceptable to use living human beings in the transdermal study. Therefore, transdermal kinetic parameters of the selected mycotoxins were obtained using human split-thickness skin in a static *in vitro* Franz diffusion cell set-up (Logan Instruments Corp., NJ, USA) with a receptor compartment of 5 mL. The experiment was replicated three times with three different skin donors ($n = 9$). Excised human skin from three female patients, who had undergone an abdominoplastic procedure, was used (44 ± 6 years old, mean ± SEM). Skin preparation was done according to the internationally accepted guidelines (OECD, 2004). Immediately after the surgical removal, the skin was cleaned with 0.01 M PBS pH 7.4 and the subcutaneous fat was removed. The skin samples were wrapped in aluminium foil and stored at −20 °C for no longer than 6 months. Just before the experiments, the skin samples were thawed and dermatomed to a pre-set thickness of 400 μm. The experimentally obtained thickness of the skin, determined using a micrometer (Mitutoyo, Tokyo, Japan), was 380 ± 8 μm (mean ± SEM, $n = 50$). Skin samples were sandwiched between the donor and the receptor chambers of the diffusion cells (0.64 cm^2 diffusion area) and held together with a clamp. The receptor compartment was filled with receptor medium (*i.e.* 1% BSA in PBS (Baert et al., 2010)), making sure all air under the skin was removed. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was continuously mixed using a Teflon coated magnetic stirring bar (400 rpm). Before starting the skin experiments, skin impedance was measured using an automatic micro-processor controlled LCR Impedance Bridge

Table 2
FDC sample stability after seven days at −35 °C (% towards $T_0 \pm$ RSD, $n = 3$).

Mycotoxin	1% BSA in PBS	70/30 (V/V) EtOH/H_2O	Pure ethanol[a]
AFB1	98.51 ± 26.76	95.69 ± 10.35	99.17 ± 17.32
OTA	108.72 ± 24.76	106.95 ± 11.73	48.40 ± 7.21
FB1	122.30 ± 15.63	107.28 ± 5.14	84.82 ± 8.12
CIT	84.90 ± 9.52	96.19 ± 12.60	100.76 ± 16.29
ZEA	89.63 ± 26.83	101.02 ± 3.52	123.11 ± 18.88
T-2	86.43 ± 8.89	114.11 ± 8.84	111.29 ± 12.33

[a] Exposed to blank skin for 12 h.

(Tinsley, Croydon, UK) to ensure that there was no skin damage (skin integrity test). Skin pieces with an impedance value below 20 kΩ were discarded and replaced. A mixture of six selected mycotoxins at an individual concentration of 1 mg/mL in 70/30 (V/V) EtOH/H_2O was topically applied to the epidermal surface of the skin (500 μL). The donor compartment was then covered with Parafilm (American National Can™, Chicago, USA) and the temperature of the receptor compartment was kept at 32 ± 1 °C by a water jacket. FDC samples of the receptor fluid (200 μL) were drawn at regular time intervals from the sample port (2, 4, 7, 10, 12, 20 and 24 h) and were immediately replaced by 200 μL fresh solution. The analytically determined mycotoxin assay values in the FDC samples were correspondingly corrected for the replenishments. At the end of the experiment (*i.e.* after 24 h), the skin surfaces were swabbed with cotton wool and the mycotoxins were extracted from the skin with ethanol to construct a mass balance: the mean percentage of each mycotoxin recovered was between 98.7 ± 6.00 and 181.0 ± 15.3, confirming the semi-quantitative validity of our data. From the skin extractions, the mycotoxin concentrations within the skin was determined, considering a skin volume of 0.02 mL (0.04 cm (skin thickness) × 0.64 cm^2 (skin surface)). Although mycotoxin metabolization in the skin has been reported, we have only assayed the unmetabolized parent mycotoxins in skin and receptor fluid.

2.5. Kinetic analysis of Franz diffusion cell data

The skin permeation parameters were calculated from the plot of cumulative amount of each mycotoxin permeated as a function of time. Steady-state flux (J_{ss}) was obtained from the slope of the linear portion of the curve divided by 0.64 to correct for the exposed skin area. The lag time (t_{lag}) was estimated by extrapolating the linear portion of the curve to the time-axis. The cumulative quantity, expressed as % of the effective dose applied, obtained after one day is the Q_{1d}. From these experimentally obtained secondary kinetic parameters, the apparent primary parameters could be calculated according to ECETOC, CEFIC (ECETOC, 1993). The permeability coefficient K_p was obtained using the following equation:

$$K_p = \frac{J_{ss}}{C_v}$$

where C_v is the concentration of each mycotoxin in the dose formulation. Consequently, the diffusion coefficient D_m and partition coefficient K_m (skin/dose-vehicle) were estimated as follows:

$$D_m = \frac{d^2}{6 \cdot t_{lag}}$$

$$K_m = \frac{K_p \cdot d}{D_m}$$

where d is the measured skin thickness in cm.

3. Results

3.1. Franz diffusion cell experiments using human skin

For the first time it is shown that, beside T-2 toxin, also AFB1, OTA, CIT and ZEA permeate the human skin when applied in 70/30 (V/V) EtOH/H_2O. Only FB1 could not be detected in the receptor fluid samples (concentration below detection limit of 10 ng/mL). All other mycotoxins confirmed the steady-state principle: after 24 h, only 0.04–2.66% of the dose applied, was cumulatively found in the receptor chamber. Fig. 1 shows the individual plots of the

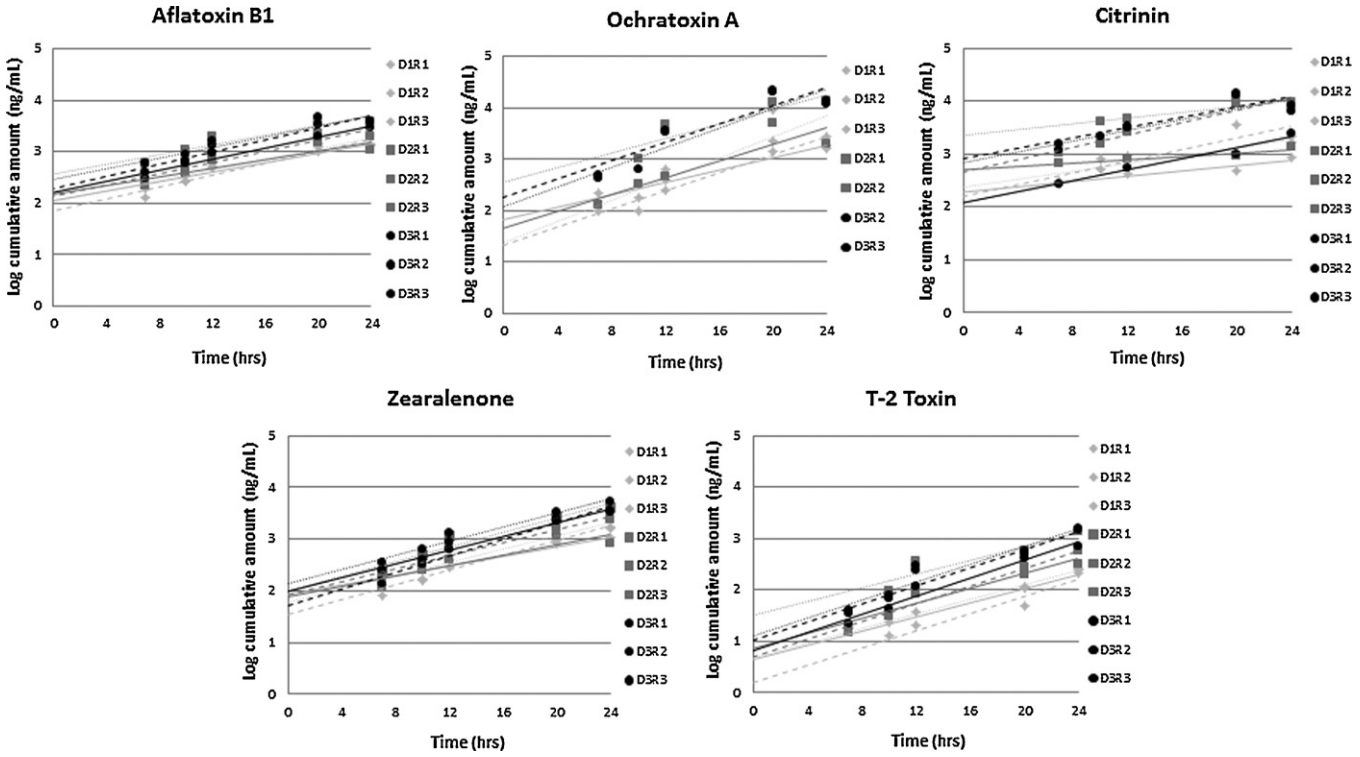


Fig. 1. Individual log/lin curves of the cumulative amount *versus* time of the investigated mycotoxins (mean ± SEM).

Table 3
Transdermal parameters for mycotoxins in 70/30 (V/V) EtOH/H_2O (mean ± SEM, n = 8–9).

Mycotoxin	Observed secondary parameters		
	J_{ss} (ng/cm^2/h)	t_{lag} (h)	Q_{1d} (%)
AFB1	213.754 ± 36.537	4.850 ± 0.363	0.468 ± 0.078
OTA	812.924 ± 216.511	7.193 ± 0.182	1.299 ± 0.381
FB1[a]	<3.255	0.000	<0.028
CIT	469.329 ± 126.038	3.561 ± 0.806	0.950 ± 0.243
ZEA	231.434 ± 43.855	6.436 ± 0.382	0.534 ± 0.102
T-2	61.941 ± 15.428	8.222 ± 0.165	0.146 ± 0.037
Mycotoxin	Apparent primary parameters		
	K_P (10^{-4} cm/h)	D_m (10^{-4} cm^2/h)	K_m
AFB1	2.113 ± 0.361	0.530 ± 0.059	0.164 ± 0.031
OTA	8.200 ± 2.184	3.358 ± 0.087	0.924 ± 0.240
FB1[a]	<0.032	–[b]	–[b]
CIT	4.667 ± 1.253	21.151 ± 12.851	0.284 ± 0.102
ZEA	2.334 ± 0.442	3.873 ± 0.291	0.252 ± 0.054
T-2	0.607 ± 0.151	2.934 ± 0.063	0.080 ± 0.021

[a] FB1 concentration in receptor fluid below LoD = 10 ng/mL.
[b] Not applicable.

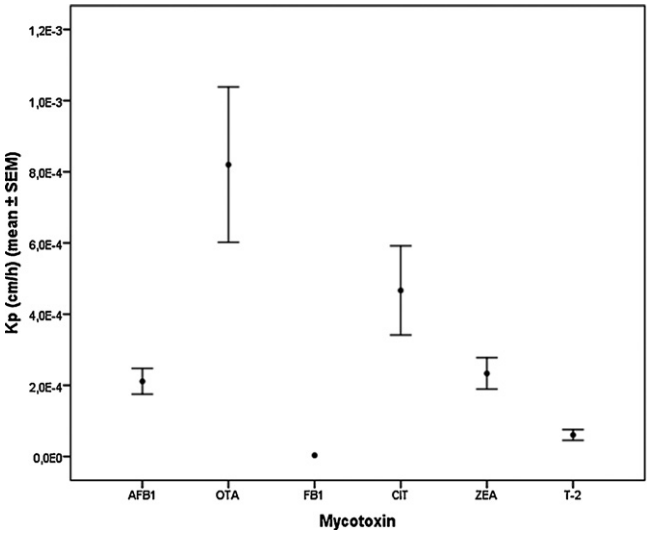


Fig. 2. Mean permeability coefficient K_p the different mycotoxins.

cumulative amounts of the mycotoxins (ng) *versus* time (h). Linear regression of the individual cumulative amounts for each mycotoxin *versus* time curves was performed in order to calculate the transdermal parameters. Their mean values (±SEM, n = 8–9) are given in Table 3.

The steady-state apparent permeability (K_p) coefficients of the individual mycotoxins are visualized in Fig. 2. OTA shows the highest permeation ($K_p = 8.20 \times 10^{-4}$ cm/h), followed by CIT ($K_p = 4.67 \times 10^{-4}$ cm/h). AFB1 and ZEA show lower, similar permeability rates ($K_p = 2.11$ and 2.33×10^{-4} cm/h, respectively). T-2 was found to have the lowest measurable permeability coefficient ($K_p = 6.07 \times 10^{-5}$ cm/h). FB1 has a K_p below $<3.24 \times 10^{-6}$ cm/h. Analysis of the skin (epidermis + dermis) after 24 h resulted in skin concentrations (mean ± SEM, n = 8) of 3.58 ± 0.25 mg/mL for AFB1, 1.89 ± 0.33 mg/mL for OTA, 15.09 ± 4.26 μg/mL for FB1, 0.70 ± 0.14 mg/mL for CIT, 0.67 ± 0.13 mg/mL for ZEA and 0.84 ± 0.14 mg/mL.

3.2. Determination of dermal daily exposure (DDE)

The DDE was estimated for three different scenarios (1) the industrial exposure of agricultural workers to grain dust contaminated with mycotoxins, (2) the industrial exposure of agricultural/food related workers to plant (extracts), fruit juices, etc. contaminated with mycotoxins, (3) the residential exposure to mycotoxin-containing dust of inhabitants of (water-damaged) houses. DDE was calculated according to the equation from OSWER (Office of Solid Waste and Emergency Response) as part of EPA (EPA, 2007):

$$\mathrm{DDE} = \frac{[\text{mycotoxin}] \cdot K_p \cdot \mathrm{SA} \cdot \mathrm{ED} \cdot \mathrm{EF} \cdot \mathrm{EV} \cdot t_{\text{event}}}{\mathrm{BW} \cdot \mathrm{AT}} \quad (1)$$

In Eq. (1), [mycotoxin] is the mycotoxin exposure concentration. Although various exposure data have been found in literature, in this risk assessment, we will limit ourselves to the central tendency exposure (CTE) approach, giving a typical or average exposure to individuals in a certain scenario (EPA, 2012). Only if the median is not available, a mean value is considered. Mycotoxin exposure concentrations are depicted in Table 4. For scenarios 1 and 3, the exposure source is contaminated dust in the air and hence the mycotoxin concentration is expressed as amount/m^3 air. This information is obtained immediately (for AFB1 and OTA) or needs to be derived from literature data reporting the amount of mycotoxin per amount of dust and the amount of dust present/m^3 environmental air. This latter was pre-defined at 7 mg dust/m^3 air (Halstensen et al., 2004) and 8.25 μg dust/m^3 air (Leese et al., 1997) for respectively the grain dust exposure in the agricultural scenario 1 and for the indoor house dust in the residential scenario 3. As exposure data used in risk assessment are very often an estimation of the real-time exposure, it is important one is aware of the fact that these exposure data need to be interpreted with caution. Moreover, the absence of exposure data does not mean that the potential risk does not exist. The apparent permeability coefficient K_p for the investigated mycotoxins is obtained from the *in vitro* transdermal FDC experiment (Table 3). The other exposure parameters are pre-defined to obtain the best estimates for three different real-life exposure scenarios. For scenario 1, the head, arms and hands were considered to be exposed, resulting in a surface area (SA) of 0.41 m^2, while for scenario 2, where only the hands are contacting the plant extracts, fruit juices, etc. an exposure area of 0.08 m^2 was taken (USEPA, 1997). In scenario 3, the head, hands, forearms, lower legs and feet, corresponding to 0.61 m^2, are assumed to be exposed to the circulating dust. In each of the scenarios, the body weight (BW) was assumed to be 70 kg (CDER, 2005). The event frequency (EV) was also fixed for all scenarios (1 event/day). Exposure duration (ED) is 25 years for the industrial conditions, while in the

Table 4
Exposure data for the three hypothetical scenarios.

Scenario	AFB1	OTA	FB1	CIT	ZEA	T-2 toxin
1[a] (ng/m^3)	421[b,12]	0.04[a]	–[c]	1.3[d]	1.4[d]	<350[e]
2 (ng/g)	81.6[f]	1.95[g]	115[h]	42.55[g]	6.15[g]	3.25[g]
3[k] (ng/m^3)	0.074[i]	0.120[i]	–[c]	–[c]	–[c]	0.018[j,l]

[a] Amount dust/m^3 air for scenario 1 is 7 mg dust/m^3 air (Halstensen et al., 2004).
[b] Selim et al. (1998).
[c] Not available.
[d] Tangni and Pussemier (2007).
[e] Nordby et al. (2004).
[f] Barkai-Golan and Paster (2008).
[g] Santos et al. (2009).
[h] Martins et al. (2001).
[i] Polizzi et al. (2009).
[j] Smoragiewicz et al. (1993).
[k] Amount dust/m^3 air for scenario 3 is 8.25 μg dust/m^3 air (Leese et al., 1997).
[l] No median but averaged value is presented.

Table 5
Estimate of DDE (ng/(kg BW day)) for the three different scenarios.

Scenario	AFB1	OTA	FB1	CIT	ZEA	T-2 toxin
Genotoxic/carcinogenic						
1	1.024×10^{-5}	3.782×10^{-9}				
2	**0.382**	0.035			–[a]	
3	5.641×10^{-9}	3.531×10^{-8}				
Non-genotoxic/non-carcinogenic						
1		1.058×10^{-8}	–[a]	1.959×10^{-7}	1.053×10^{-7}	$<6.890 \times 10^{-6}$
2	–[a]	0.099	<0.021	1.233	0.089	0.012
3		8.240×10^{-8}	–[a]	–[a]	–[a]	9.290×10^{-10}

DDE in bold exceeds the NCRI.
[a] No data available.

domestic scenario, 30 years were assumed (EPA, 2007). Averaging times (ATs) for non-carcinogenic chemical exposures are equivalent to the ED (USEPA, 1997) *i.e.* 25 years (9125 days) for industrial and 30 years (10,950 days) for residential conditions. The AT for carcinogenic chemical exposures is 70 years for all exposed individuals (USEPA, 1997). The exposure frequency (EF) is supposed to be 250 and 350 days/year (EPA, 2007) with an event duration (t_{event}) of 8 and 10 h/event considering the industrial and residential exposure, respectively. From Eq. (1) the DDE for each scenario and for each of the investigated mycotoxins is calculated and depicted in Table 5.

4. Discussion

For the first time, quantitative transdermal parameters were obtained using human skin which is the golden standard. Although different research results are indicative for penetration into skin (Albarenque and Doi, 2005; Albarenque et al., 1999; Antony et al., 2002; Bhavanishankar et al., 1988; Blaylock et al., 1993; Bunner et al., 1988; Dimitri and Gabal, 1996; Hsia et al., 2004; Konoshima et al., 1999; Kumar et al., 2011; Lambert et al., 1995; Liu et al., 1999; Rea et al., 2003; Reboux, 2006; Saxena et al., 2009; Scheie et al., 2003; Wei et al., 1972), currently, the dermal route for mycotoxins was hardly investigated, with only limited fragmentary studies, often lacking quantitative data. However, this exposure route is non-negligible, and can result in local as well as systemic effects (Degen, 2011). Possibly, contaminated food and feeds, and even herbal preparations used in cosmeceuticals, are manipulated by both processors and traders, but also by the consumer. As it was found that air and dust particles can contain mycotoxins, especially in grain storage (Halstensen et al., 2004; Selim et al., 1998; Tangni and Pussemier, 2007) and humid indoor places where fungi are present (Polizzi et al., 2009; Smoragiewicz et al., 1993), these particles cannot only be inhaled but can also end up at the skin. Moreover, numerous other contaminated surfaces, like surfaces where contaminated air particles land or surfaces containing fungi (even sheets and pillows), can contact the skin.

4.1. Comparison of permeability data with in silico and literature information

Comparing our experimentally obtained K_p data with the *in silico* Potts and Guy model (Potts and Guy, 1992), similar results were obtained for AFB1, CIT and T-2 (see Tables 1 and 3), while the experimentally obtained $K_{p,OTA}$ and $K_{p,ZEA}$ are 10, respectively 40, times higher than the *in silico* prediction. This deviation between the experimental and *in silico* predicted values is not uncommon: it has been recognized that *i.a.* the dose formulation may significantly influence the transdermal behaviour of compounds, depending on solvent and compound (Boonen et al., 2010). Where the Potts and Guy model considers the aqueous permeability coefficient of a single component, we investigated a mixture of mycotoxins in a 70% ethanolic formulation. The choice of the vehicle is highly important as it significantly influences the transdermal behaviour of permeating compounds (Baert and De Spiegeleer, 2011; Boonen et al., 2010; Williams and Barry, 2004). The ethanolic–water mixture was chosen as solvent, not only for solubility reasons, but also as a reasonable worst case model formulation seen "penetration enhancers" like ethanol are most probably also present in real-life. Caution must also be taken when cleaning skin surfaces exposed to mycotoxins, as certain decontaminating solvents and excessive skin rubbing may enhance skin absorption (Kemppainen, 1988). EtOH/H_2O mixtures have penetration promoting effects by extraction of the lipids and changing the skin's protein conformation (Artusi et al., 2004). Other mycotoxin exposure vehicles can also contribute to their dermal absorption. For example, contaminated nano- and even microsized air/dust particles might function as transport carriers for mycotoxins. The skin penetrating properties of nano- and microparticles into the viable skin layers and even into the dermis have been established (Boonen et al., 2011;

Crosera et al., 2009). Moreover, to approximate the in-use conditions, a mixture of mycotoxins was investigated. The possible mutual effect of these mycotoxins on the transdermal behaviour can influence the outcome (Riviere and Brooks, 2005; van der Merwe and Riviere, 2005). Thus, a penetrating-enhancing effect cannot be excluded. Again, this also reflects the real-life situation where quite often, a mixture of mycotoxins is found (Flajs et al., 2011; Flajs and Peraica, 2009; Grenier and Oswald, 2011; Pfohl-Leszkowicz and Manderville, 2007). In this context, it was suggested that combination effects of mycotoxins present in mouldy foods, acting via different mechanisms, may have an impact on the overall cancer hazard to humans (Knasmuller et al., 2004). As the co-occurrence of mycotoxins is well-documented, the toxicological interactions of mycotoxins (Grenier and Oswald, 2011) are also relevant in terms of risk assessment. Therefore, hazard characterization of mixtures of mycotoxins is warranted.

Presently, there are no studies on skin absorption of ochratoxins, CIT and ZEA (Braese et al., 2009). The permeation of the water-soluble FB1 through skin was questioned (Flynn, 1985), which is confirmed with our data. Historical quantitative skin permeation data are present for AFB1 and T-2 (see Supplementary Table S1). Riley et al. (1985) found that the dermal permeability of AFB1 in MeOH through human epidermal skin is slow but insignificant. From their flux curves over 24 h applying a dose solution of 0.65 mg/mL in an occluded way, a K_p value of 6.09×10^{-7} cm/h could be derived. In our study, a K_p of 2.11×10^{-4} cm/h was found. Factors like the skin thickness and formulation could be the reason for these different observations (Kemppainen, 1988). Literature data of T-2 are more consistent with our findings. A flux value of 24.2 pg/(cm^2 h), with 20.2 ng T-2/μL MeOH applied on human frozen skin (Kemppainen et al., 1986), corresponding to a K_p of 1.20×10^{-6} cm/h is similar to our observation ($K_p = 6.07 \times 10^{-5}$ cm/h).

4.2. Local skin concentrations and effects

For some of the investigated mycotoxins, local skin concentrations after 24 h exposure are high, compared to the dermally applied mycotoxin concentration (1 mg/mL): 3.58 mg/mL AFB1 and 1.89 mg/mL OTA were found in skin. For CIT, ZEA and T-2 toxin, concentrations between 0.67 and 0.84 mg/mL were determined. For FB1, the skin concentration is an order of magnitude lower than the skin concentrations of the other investigated mycotoxins (15.09 μg/mL). The high local concentrations of AFB1 and OTA can be due to the skin reservoir function for these mycotoxins. Although our data did consider a skin thickness of ± 400 μm, encompassing the stratum corneum (SC), epidermis and part of the dermis, it has already been mentioned that the SC can act as a reservoir for T-2 toxin (Bunner et al., 1989; Pang et al., 1987). The log P of the mycotoxins (Table 1) could partly be responsible for the skin reservoir properties: the higher the log P, the higher the distribution of the mycotoxin towards the upper skin layer *i.e.* the SC. Indeed, the log P for the mycotoxin with the lowest skin concentration, FB1, was estimated to be −0.78, while the log P for the mycotoxin with the highest local concentration, OTA, was 4.27. Moreover, log P is not only mechanistically determines local skin concentrations, but is also an important physicochemical descriptor used in several models predicting the transdermal permeability (Wilschut et al., 1995). However, the skin is a heterogeneous membrane, having different layers from very lipophilic (SC), over lipophilic/hydrophilic (epidermis) to more hydrophilic (dermis) strata. As the log P values reflect the affinity for the SC, the formulation-contacting layer contributing for less than one tenth of the integral skin thickness, log P values are not the only descriptors for explaining the skin mycotoxin concentration. After overcoming the first barrier layer (SC) for which a sufficiently high log P is required, the dermally applied compounds need to penetrate into and through deeper skin layers, for which the log P is ideally between 1 and 3 (Brown et al., 2006). Where very lipophilic compounds will stay in the SC, more hydrophilic ones will distribute to the underlying, more hydrophilic strata of the epidermis and dermis. It is also possible that mycotoxins bind to the high amount of proteins, like collagen, present in the dermis. Although there is no information about the precise cellular local concentration of the different mycotoxins in the skin, local skin effects are also to be expected. Apoptosis of epidermal cells and development of skin tumours are described skin effects after dermal mycotoxin exposure. Application of 1 mg/mL AFB1 twice within 48 h resulted in intra-epidermal vesicles containing a leukocytic exudate, while higher concentrations (18 mg/mL) yielded epidermal cell death (Joffe and Ungar, 1969). FB1 shows apoptotic and anti-proliferative effects in neonatal human keratinocytes (NHKs) with an IC_{50} of 10^{-5} M corresponding to 36 μg/mL (Tolleson et al., 1996). The apoptotic process of FB1 passed different stages within six days. After a decreased NHKs expansion, cell membranes lost their integrity with omission of metabolic competency and end up in cellular swelling and cell death. The inhibition of ceramide synthase is believed to be responsible (Melchior et al., 1974). BALB/c mice keratinocyte cell lines (C5-O) were found to be even more sensitive to ZEA (IC_{50} = 24.1 μg/mL) than to FB1 (IC_{50} > 100 μg/mL) (Cetin and Bullerman, 2005). The increased apoptosis in C5-O cell by ZEA (40 μM or 13 μg/mL) which occurs already after 24 h is due to DNA interference and induction of reactive oxygen species (ROS) (Ben Salah-Abbes et al., 2010). Analysis of several oxidative stress markers and up-regulation of DNA damage related proteins suggest that generation of ROS is also the responsible mechanism for epidermal apoptosis by CIT. Dermal application of 250 μg/mL CIT on mice skin up to 72 h in an *in vivo* assay, leads to a 25-fold increased apoptosis compared to a control (Kumar et al., 2011). The mechanism for T-2 related apoptosis of basal epidermal keratinocytes is somewhat different. The induction of TNF-α and TGF-β1 mRNAs as well as the apoptosis related genes *c-fos* and *c-jun* were found to resulted in basal cell apoptosis within 24 h after dermal application (0.5 mg/mL) (Albarenque et al., 2000, 2001). Moreover, the increased TGF-β1 expression functions as chemoattractant for mast cells, resulting in inflammatory responses of the dermis (Doi et al., 2008). On the contrary, T-2 toxin (150 μg/mL) inhibits dermal contact hypersensitivity, reflected in a 44% reduction of ear swelling in BALB/c mice, when it is applied within 1 h after dermal dosing with oxazolone (Blaylock et al., 1993). The underlying mechanism is the inhibition of protein synthesis and antigen presentation by epidermal Langerhans cells. The inhibition of the skin immune system after 24 h, can lead to serious health effects, making persons more susceptible to infections or skin cancers (Blaylock et al., 1993). Indeed, the skin tumour initiating and promoting properties of AFB1, FB1 and T-2 toxin were demonstrated. After a single dermal dose (250 μg/mL) of AFB1 followed by repeated topical application of the tumour promoter 12-tetradecanoyl phorbol myristate acetate (TPMA), all mice developed squamous cell carcinomas after a latency time of 13 weeks (Rastogi et al., 2006). However, AFB1 showed no tumour promoting effects if 12.5 μg/mL is given twice weekly, 24 weeks, after a dimethylbenzanthracene (DMBA) initiator dose, nor complete carcinogenicity (50 μg/mL given twice weekly, 24 weeks). On the contrary, FB1 and T-2 showed weak initiating activity, but strong skin tumour promoting effects in mice (Hirokazu Fukuda et al., 1996; Konoshima et al., 1999; Lindenfelser et al., 1974; Marasas et al., 1969; Yang and Xia, 1988). For T-2 toxin, 9–18% of the mice dermally treated with T-2 toxin as tumour promoter (after single administration of the initiator DMBA) developed skin papillomas, while 2–5% developed skin carcinoma (Lindenfelser et al., 1974; Marasas et al., 1969; Yang and Xia, 1988). Due to the reservoir properties of the skin (Baert and De Spiegeleer, 2011), persons not only

Table 6
Hazard characterization (ng/(kg BW day)) data for the investigated mycotoxins.

Mycotoxin	NCRI/TDI[a]	Main systemic effect
Genotoxic/carcinogenic		
AFB1	0.0138[b]	Hepatocarcinogen
OTA	4–10[c,d]	Nephrocarcinogen
Non-genotoxic/non-carcinogenic		
OTA	3–53[c,e,f]	Nephrotoxic
FB1	2000[g]	Nephrotoxic and hepatotoxic
CIT	400[h]	Kidney injury
ZEA	200–500[i]	Hormonal effects
T-2	60–700[j,k]	Immune effects

[a] NCRI for genotoxic and carcinogenic mycotoxins; TDI for non-genotoxic and/or non-carcinogenic mycotoxins.
[b] Derived from Benford et al. (2010): $BMDL_{05}$/5000.
[c] Kuiper-Goodman et al. (2010).
[d] Haighton et al. (2012).
[e] JECFA (1991).
[f] Haighton et al. (2012).
[g] SCF (2000b).
[h] Estimated TDI: 10 mg/(kg BW day) (LOAEL) (Shinohara et al., 1976) × 25,000^{-1} (SF_1 = 5, SF_2 = 10, SF_3 = 10, SF_4 = 5, SF_5 = 10) (EMA, 2011).
[i] SCF (2000a).
[j] SCF (2001).
[k] EC (2006).

chronically, but also occasionally exposed to the investigated mycotoxins, have an increased risk for epidermal apoptosis, skin cancers and immune related diseases.

4.3. Risk assessment of mycotoxins after dermal exposure

For non-thresholded human carcinogens, several metrics can be used (TTC, ALARA, linear extrapolation, margin of exposure (MoE), negligible cancer risk intake (NCRI)) (Barlow et al., 2006; Kuiper-Goodman et al., 2010; SCHER/SCCP/SCENIHR, 2009). The NCRI is the exposure associated with a risk level of 1 per 100,000 exposed individuals (Kuiper-Goodman et al., 2010). As the NCRI (expressed as ng/kg BW day) is a clear concept, we used this risk-metric for the genotoxic and carcinogenic mycotoxins AFB1 and OTA. For non-genotoxic or DNA-nonreactive carcinogenic compounds, other threshold values can be applied with the tolerable daily intake (TDI), as most often used for mycotoxins by the European commission (EC, 2006; SCF, 2000a,b). The TDI is an estimation of a daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime and can be derived from the NOAEL, LOAEL, or benchmark dose (BMD), with safety or uncertainty factors (SF, UF) generally applied to reflect limitations of the data used (EMA, 2011; IRIS, 1993). Table 6 summarizes the hazard characterization from literature of the mycotoxins investigated in current study.

For AFB1, different risk assessment parameters have been derived (NOAEL, LOAEL, T25, $BMD(L)_{01,05,10}$, T_{50}) (Benford et al., 2010; Kuiper-Goodman, 1990). We have used the benchmark dose confidence lower limit at 5% ($BMDL_{05}$), which is likely to cause not more than 5% cancer incidence, as point of departure. The $BMDL_{05}$ was derived through modelling the tumour incidence data of Wogan et al. (Wogan et al., 1974), using the multistage method ($BMDL_{05}$ = 0.069 μg/kg BW day) (Benford et al., 2010). Dividing the $BMDL_{05}$ (5/100) by 5000, gives the exposure (NCRI = 0.0138 ng/kg BW day). For OTA, the NCRI at a risk level of 1:100,000 was derived from the TD_{05} and ranged to be 4–10 ng OT/kg BW day (Haighton et al., 2012; Kuiper-Goodman et al., 2010).

The TDI for the nephrotoxicity of OTA ranged from 3 to 53 ng/(kg BW day). Originally, JEFCA derived an OTA TDI (LOAEL/UF) for nephrotoxicity of 16 ng/(kg BW day) (JECFA, 1991). In 2010, this TDI was revised by replacing the LOAEL (8 μg/(kg day)) by the benchmark dose (BMD_{10} = 1.56 μg/(kg BW day)) and by increasing the uncertainty factor of 450–500. This lowers the TDI for renal damage from 16 ng/(kg BW day) to 3 ng/(kg BW day) (Kuiper-Goodman et al., 2010). However, Haighton et al. (2012) questioned the interspecies uncertainty factor of 15 and 25 used by JEFCA and Kuiper-Goodman et al. and changed it to a factor of five, resulting in a TDI of 53 and 15.6 ng/(kg BW day), respectively. For FB1, a TDI of 2 μg/(kg BW day) (derived from the NOAEL of 0.2 mg/(kg BW day) with UF of 100) was set (SCF, 2000b). No TDI is available for the non-genotoxic, non-carcinogenic citrinin. Therefore, we used the thresholded nephrotoxicity of citrinin in rats as response for its safety assessment (Table 6). In the study of Shinohara et al. (1976), LOAEL levels, resulting in kidney injury but without tumour induction, are reported (Shinohara et al., 1976). Correcting the LOAEL of 10 mg/(kg BW day) with a global uncertainty factor of 25,000 (5 [interspecies difference between rats and humans] × 10 [inter-individual differences] × 10 [for short-term exposure toxicity studies] × 5 [toxic severity] × 10 [use of LOAEL instead of NOAEL] (EMA, 2011), resulted in a TDI of 0.4 μg/(kg BW day). JEFCA established a TDI for the hormonal effects of ZEA of 0.5 μg/(kg BW day), while the European commission fixed the TDI on 0.2 μg/(kg BW day) (SCF, 2000a). The TDI of the immune-associated effects of T-2 toxin are estimated between 0.06 and 0.7 μg/(kg BW day) (EC, 2006; SCF, 2001).

In order to investigate the safety concern of the mycotoxins, the calculated human DDEs (Table 5) are compared with the negligible cancer risk intake (NCRI) for the DNA-reactive genotoxic and carcinogenic mycotoxins AFB1 and OTA, and compared with the tolerable daily intake (TDI) of the thresholded mycotoxins (*i.e.* OTA, FB1, CIT, ZEA and T-2) for all three exposure scenarios (Table 6). Only the estimated DDE for AFB1 (0.382 ng/(kg BW × day)) in scenario 2 exceeds the NCRI of AFB1 (0.0138 ng/(kg BW × day)). All other DDEs are below the NCRI or TDI, for carcinogenic and non-carcinogenic mycotoxins, respectively. Hence, dermal contact with mycotoxin contaminated dust/air particles in both agricultural (scenario 1) and residential (scenario 3) environments do not impose significant health concerns. Agricultural workers being in contact with solutions containing AFB1 however (scenario 2), do have an increased risk to develop liver cancer. Nevertheless, for OTA, no significant cancer risk is posed in this scenario. In scenario 2 also no threshold related renal, hormonal or immune effects, with OTA, FB1, CIT; ZEA and T-2 toxin exposure are expected, respectively. It is important to emphasize that the used exposure data for this risk assessment need to be interpreted with caution. For each individual scenario, real exposure parameters can vary and thus deviate from the used estimations. Therefore, if reliable site-specific data are available, these ones need to be used to provide the best risk estimate.

Some reflections are to be made for the correct interpretation of the results. Apart from the default exposure values used in the different scenarios, there is also the fact that our K_p values are obtained from infinite dosing. As for several of the investigated mycotoxins, no quantitative dermal absorption data were available, we applied an infinite dose approach as worst case scenario. In risk assessment, ideally, real-life exposure conditions using concentration depleted finite doses in environmental vehicles should be considered to achieve dermal absorption estimates. In practice, however, initial estimates for the dermal absorption use infinite doses (WHO, 2006). Moreover, recent investigations are exploring models to link permeability coefficients measured under infinite exposure conditions to conditions more typical for occupational exposure (Kruse et al., 2007).

In this study, only a single mycotoxin dose was applied on the skin. In real-life exposure, contaminated environments (like workplaces or contaminated houses), no single but multiple, often chronic, exposure to mycotoxins occurs. The influence of

multiple exposure on the alteration of the dermal absorption is still a controversial subject (WHO, 2006). Where some researchers found lower systemic concentrations after multiple-dose administration compared to single application due to redistribution of penetrating compounds from the systemic circulation to the skin tissue (Dehghanyar et al., 2004), others found no differences between single and multiple dosing regimes (Wester et al., 1994).

It is also clear that the health risks are increased in susceptible populations with diminished skin barrier function: premature infants, young children, elderly persons and patients with skin diseases like psoriasis. Additionally, compromised skin due to chemical or mechanical damage, shows a higher permeability for mycotoxins. In a comparative *in vivo* study where T-2 toxin was topically applied on abraded *versus* intact rat skin (0.28 mg/mL), 50% of the dose permeated through abraded skin after 1.5 h, while only 2% of the dosed permeated through the intact skin (Solberg et al., 1990).

Finally, mycotoxins may be subjected to *in vivo* skin metabolism. Both, phase I and phase II enzymes were found in the skin (Roberts and Walters, 2007). These enzymes can influence the *in vivo* toxicity and percutaneous absorption of mycotoxins. AFB1 is bio-activated to the acutely toxic and carcinogenic AFB1-8,9-epoxide by CYP subfamilies 1A, 2B, 2C, and 3A. Detoxification of the epoxide occurs with the glutathione transferase or hydrolyze (Hussein and Brasel, 2001). OTA is metabolized by CYP 2C9 and 3A4 into hydroxylated metabolites. Undergoing the 2C9 metabolization, an increased cytotoxicity has been observed, while CYP3A4-derived biotransformation products are less toxic (Doorten et al., 2004). FB1 can be metabolized by esterases. The totally hydrolyzed FB1, aminopentol, has the same toxic effects as FB1, but is even more potent (Varga and Toth, 2005). The skin metabolism of T-2 toxin, diacetoxyscirpenol (DAS) and verrucarin A (VCA) has specifically been studied. For DAS and VCA, about 50% and 80% was metabolized in skin, respectively (Kemppainen et al., 1987a). T-2 toxin has been metabolized extensively (up to 90%), with HT-2 toxin as the major metabolite (80%) in human skin, followed by T-2 tetraol, T-2 triol, which all are less toxic than T-2 toxin itself (Kemppainen, 1988; Kemppainen et al., 1987b). Moreover, 3′-hydroxy-T-2, 3′-hydroxy-HT-2, 3′-hydroxy-T-2 triol, and actyl-T-2 toxin, were tentatively identified in human skin (Wu et al., 2010). The hydroxylation of T-2 toxin is an activation pathway, as 3′-hydroxy-T-2 is found to be more toxic than T-2 toxin and hence exerts even more toxic effect to humans than T-2 toxin itself (Yoshizawa et al., 1984). Some MTs can even induce or decrease the expression of enzymes in the skin, possibly resulting in an altered metabolism of accompanying mycotoxins. T-2 toxin for example decreases the CYP P-450 expression of 1A1, 1A2, 2A1, 2B4, but not of 2C3 and 3A6 in rat liver (Guerre et al., 2000), while AFB1 significantly induces the cutaneous CYP 1A enzymes (Rastogi et al., 2006). It is thus clear that the mutual influence of dermal co-occurring mycotoxins can affect their potency and toxicological concern, which requires further investigation.

Our results, indicating that mycotoxins can penetrate into and through the skin, lead to the awareness of health risks for people who are dermally exposed to mycotoxins. Although for most of the mycotoxins it was found that they do not pose a general health risk in several real-life scenarios, it is recommended whenever possible, to avoid or at least limit the dermal exposure of these mycotoxins, especially when people are in contact with solutions containing the very genotoxic carcinogen AFB1.

5. Conclusions

For the first time, quantitative skin permeability data of six selected mycotoxins were obtained by an *in vitro* FDC infinite dose approach using dermatomed split-thickness human skin. Based on hazard characterization of the selected mycotoxin, risk assessment revealed that a cancer health risk is present after dermal exposure to AFB1, but not to OTA. Moreover, non-cancer related health effects are not expected after dermal exposure to FB1, CIT, ZEA and T-2 toxin. However, seen extremely toxic mycotoxins can indeed permeate the skin, it is recommended to limit their dermal exposure.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tox.2012.06.012.

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